

Protocol for passaging of WJ-MSC/TERT273

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Designation: WJ-MSC/TERT273, human Wharton's Jelly derived mesenchymal stem cells

Growth medium: Mesencult™ ACF Plus Culture Kit (STEMCELL TECHNOLOGIES) supplemented with GlutaMAX™ and G418:

Final components:

Mesencult™ ACF Plus medium (STEMCELL TECHNOLOGIES, Cat# 05448)
Mesencult™ ACF Plus 500x supplement (STEMCELL TECHNOLOGIES, Cat# 05448)
Animal Component-Free Cell Attachment (STEMCELL TECHNOLOGIES, Cat# 05448)
2 mM GlutaMAX™ (Gibco, Cat# 35050-038, ready-to-use)
200 µg/ml G418 (InvivoGen, Cat# ant-gn5, ready-to-use, 100 mg/ml)

- take one bottle of Mesencult™ ACF Plus medium (500 ml)
- add 1 ml of Mesencult™ ACF Plus 500x supplement
- add 5 ml of GlutaMAX™
- add 1 ml of G418 stock solution
- mix properly
- store at 4°C for a maximum of 4 weeks
- temper the medium to room temperature (not 37°C) before use

Coating: ACF Cell Attachment Substrate

The coating solution is prepared by mixing the following components:
Animal Component-Free Cell Attachment (Cat# 05448, stored at 4°C)
PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at RT)

For coating of a T25 roux flask proceed as follows:

- transfer 1.8 ml of PBS to a sterile tube, add 6 µl of ACF Cell Attachment Substrate and gently mix
- transfer the 1:300 diluted ACF substrate (1.8 ml) to a T25 roux flask
- completely wet the surface of the culture flask
- incubate at room temperature for at least 2 hours
- NOTE: coated flasks can be stored for up to 3 days at 4°C – the flasks must be sealed with Parafilm to prevent evaporation – before use let the flask warm up at room temperature for 30 min
- remove excess of coating solution
- wash the flask once with PBS (160 µl/cm²)

	<ul style="list-style-type: none">- remove PBS completely- use culture flask immediately for seeding of cells, the surface must not to dry out
Additional reagents:	PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at 4°C) CTS™ TrypLE™ Select Enzym (Gibco, Cat# A1285901, ready-to-use, stored at 4°C)
Passaging of cells:	<ul style="list-style-type: none">- remove and discard the culture medium- wash the cells twice with PBS (each 160 µl/cm²), remove PBS completely- add CTS™ TrypLE™ Select Enzym solution (20 µl/cm²), make sure that all cells have been in contact with this solution- incubate the culture flask at 37°C for approximately 2-3 min- observe cell detachment under an inverted microscope- as soon as all cells are detached (if necessary, agitate the cells by gently hitting the flask), add growth medium (about 160 µl/cm²)- centrifuge at 300 g for 5 min- discard the supernatant, resuspend the cell pellet in the remaining droplet and add growth medium (about 160 µl/cm²)- transfer appropriate aliquots of the cell suspension to pre-coated culture vessels supplemented with growth medium (final volume of 240 µl/cm²)- a split ratio of 1:3 to 1:4 twice a week is recommended (after having reached about 80-90 % confluence)- cultivate cells at 37°C in a humidified atmosphere with 5% CO₂
Related products:	EV-WJ-MSC/TERT273, extracellular vesicles from WJ-MSC/TERT273 cells (Evercyte, Cat# EV-059-0273) ASC/TERT1, adipose-derived mesenchymal stem cells (Evercyte, Cat# CHS-001-0005)